Version With Markings to Show Changes Made

Claim 1 (amended) An optical antioxidant sensing process for measuring the effectiveness of a nutritional formulation by calculating the free radical scavenging efficiency of (a) the nutritional formulation when encountering reactive oxygen radical species in a medium comprising the steps of:

introducing an organic dye reagent that reacts with oxygen radicals to said medium to chemically tag said oxygen radicals in said medium;

detecting and measuring the population of said tagged oxygen radicals using an optical fiber sensor;

introducing a nutritional formulation with antioxidant properties to said medium;

detecting and measuring the relative population of said tagged oxygen radicals in said medium using said optical fiber sensor; and

NAB calculating the free radical scavenging efficiency of said nutritional formulation using said oxygen radical population measurements; and assaying the free radical scavenging effectiveness of the nutritional formulation.)

Claim 4 (amended) An optical antioxidant sensing process for measuring the effectiveness of a nutritional formulation by calculating the free radical scavenging efficiency of (a) the nutritional formulation when encountering reactive oxygen radical species in a medium comprising the steps of:

introducing an organic dye reagent that reacts with oxygen radicals to said medium to chemically tag said oxygen radicals in said medium;

introducing an oxygen catalyst promoter to said medium to increase oxidative activity;

detecting and measuring the population of chemically tagged oxygen radicals in said medium using an optical fiber sensor;

introducing a nutritional formulation with antioxidant properties to said medium;

detecting and measuring the relative population of said chemically tagged oxygen radicals in said medium using said optical fiber sensor; and

calculating the free radical scavenging efficiency of said nutritional formulation using said oxygen radical population measurements; and assaying the free radical scavenging effectiveness of the nutritional formulation.

Claim 9 (amended) An optical antioxidant sensing process (to measure the free radical scavenging efficiency of nutritional formulations) for comparing the efficiency of a food-based antioxidant to an isolated form of the antioxidant comprising:

forming a control group (including) comprising a medium (that contains) with tagged fluorescent oxygen (radicals) radical cells;

incubating a first portion of said control group with a (first) sample of a food-based source nutritional formulation having a key antioxidant (properties) ingredient;

incubating a second portion of said control group with a (second) sample of (said) a nutritional (formulation) supplement having said key antioxidant (properties) ingredient in isolated form;

measuring the free radical scavenging activity of said (first sample in said) incubated first portion of said control group using an optical fiber sensor;

measuring the free radical scavenging activity of said (second sample in said) incubated second portion of said control group using (said) an optical fiber sensor; and

(assaying) comparing the relative antioxidant capacity of said (first and said second samples)food-based source nutritional formulation having a key antioxidant ingredient and said nutritional supplement having said key antioxidant ingredient in isolated form.

Claim 10 (amended) The optical antioxidant sensing process of claim 9 wherein said (first sample comprises a food-based source of a key phytonutrient with antioxidant capabilities) key antioxidant ingredient of said food-based source nutritional formulation having a key antioxidant ingredient is a phytonutrient with antioxidant properties.

Claim 11 (amended) The optical antioxidant sensing process of claim 9 wherein said (first sample comprises a vitamin with antioxidant capabilities such as wheat germ oil) key antioxidant ingredient of said food-based source nutritional formulation having a key antioxidant ingredient is a vitamin complex with antioxidant properties.

Claim 12 (amended) The optical antioxidant sensing process of claim 9 wherein said (second sample comprises a key phytonutrient with antioxidant capabilities in isolated form) key antioxidant ingredient in isolated form is a phytonutrient with antioxidant properties.

Claim 13 (amended) The optical antioxidant sensing process of claim 9 wherein said (second sample comprises a vitamin with antioxidant capabilities in isolated form) key antioxidant ingredient in isolated form is a chemical with antioxidant properties.

Claim 14 (amended) The optical antioxidant sensing process of claim 9 wherein said (second sample comprises wheat germ oil) sample of a food-based source nutritional formulation having a key antioxidant ingredient comprises wheat germ oil.

Claim 15 (amended) The optical antioxidant sensing process of claim 9 wherein said (second antioxidant sample is an isolated form of vitamin E proven to have antioxidant activity) key antioxidant ingredient in isolated form is vitamin E.

Claim 16 (amended) The optical antioxidant sensing process of claim 9 wherein said (second antioxidant sample is Trolox) key antioxidant ingredient in isolated form is Trolox.

Claim 17 (amended) A process for measuring antioxidant activity in an in-vitro model (mimicking the gastrointestinal tract) of a gastrointestinal tract comprising the steps of:

introducing a functional food-based antioxidant sample to a first vessel containing ingredients (that mimic the environment) in a stomach segment of said gastrointestinal tract;.

pumping the resultant solution into a second vessel containing ingredients (that mimic the environment) in a small intestine segment of said gastrointestinal tract;

(introducing a pancreatic fluid solution to said second vessel to further mimic the environment of said small intestine segment;)

(introducing a bile salt solution to said second vessel to further mimic the environment of said small intestine segment;)

pumping the resultant solution into a third vessel containing ingredients (that mimic the environment) in a large intestine segment of said gastrointestinal tract; and

assaying solutions from said vessels (using the optical antioxidant sampling process of the invention) by introducing an organic dye reagent that reacts with oxygen radicals to each vessel to chemically tag said oxygen radicals and detecting and measuring the population of said tagged oxygen radicals using an optical fiber sensor to determine the solution's relative intracellular effects on free radicals in said gastrointestinal tract.

Claim 18 (new) The process of claim 17 wherein said ingredients in a small intestine segment of said gastrointestinal tract include a pancreatic fluid solution.

Claim 19 (new) The process of claim 17 wherein said ingredients in a small intestine segment of said gastrointestinal tract include a bile salt solution.

Claim 20 (new) The process of claim 17 wherein said ingredients in a stomach segment of said gastrointestinal tract have an acidic pH within the pH range found in a stomach segment of a gastrointestinal tract and said ingredients in a small intestine segment of said gastrointestinal tract have an alkaline pH within the pH range found in a small intestine segment of a gastrointestinal tract.

Remarks Section

With regard to examiners basis for the U.S.C. 103(a) rejection of claims 1-3 and 9-16 in view of the cited prior art references, i.e. U.S. Patent 5, 939,395 issued to Yu et al. and U.S. Patent 4,573,761 issued to McLachlan, there is no motivation or suggestion to modify or combine the references to teach the process of the invention for measuring the effectiveness of a nutritional formulation.

Yu et al. evaluates antioxidative activity by adding DCFHDA to a mixture containing an antioxidant sample, a rat brain homogenate, ethanol, esterase, and water (Column 5, lines 31-35). The fluorescence intensity of the mixture is then measured with a spectrofluorometer and the concentration of DCF is determined by comparison to a standard DCF curve that was constructed by plotting the fluorescence activity of samples of DCF having known concentrations. The total antioxidant activity of the mixture is then calculated by comparing the suppression rate of the sample against a control (Column 5, lines 39-50).

In contrast, the process of the invention initially adds H_2DCFDA to a medium containing live yeast cells or cell-cultured cells, without an antioxidant sample, to first identify and tag the specific oxidative species present in the medium. As stated in the specification (Paragraphs 18-19), the optical antioxidant sensing process to evaluate the effectiveness of a nutritional formulation begins with a medium having intracellular oxidation processes that include oxygen radicals into which an organic dye reagent (H_2DCFDA) is introduced (Paragraph 24) and the population of tagged oxygen radicals is measured using an optical fiber sensor (Paragraph 20).

The antioxidant sample to be analyzed is then introduced to the medium and the fate of the tagged oxygen radicals is detected using an optical fiber sensor (Paragraph 21).

The unique process of the invention uses an H_2DCFDA intracellular probe (Paragraph 25) and an optical fiber sensor (Paragraph 13) to measure the free radical scavenging capability of specific antioxidant fortified cells in order to evaluate the effectiveness of a given nutritional formulation (Paragraph 0018). The Yu et al. reference measures total antioxidant activity of a mixture containing an isolated

antioxidant but does not identify the intracellular antioxidant activity of specific antioxidant fortified cells.

Further, Yu et al. expressly discloses that the fluorescence intensity of the mixture is measured with a spectrofluorometer. The process of the invention uses an optical fiber sensor to measure the population of tagged oxygen radicals in order to determine the free radical scavenging capability of specific antioxidant fortified cells. An optical fiber sensor provides a more sensitive and accurate measurement than a spectrofluorometer as the bandwidth of light produced by a spectrofluorometer is typically greater that the single wavelength light used in an optical fiber sensor.

Regarding the examiners rejection of Claims 4-8 under 35 U.S.C. 103(a), the process of the invention expressly discloses the use of an oxygen catalyst promoter before measurement of oxygen radicals (Para 33). The specification also discloses that the promoter can be used as a control to show that the probe is converting from H_2DCF to DCF fluorescent (Para 0030). Yu et al. does not expressly disclose the addition of an oxygen catalyst promoter before measurement of oxygen radicals nor that the promoter can be used as a control to show that the probe is converting from H_2DCF to DCF.

Further, U.S. Patent 6,051,571 issued to Kelleher et al. teaches the addition of ferrous iron and tert-butyl hydroperoxide (Column 28, Lines 65-67) to add a radical generating system to a furan nitrone compound (Column 2, Lines 14-16) and to determine the ability of furan nitrones to trap free radicals (Column 28, Lines 54-59). In contrast, the process of the invention specifically teaches the addition of a promoter such as H_2O_2 with cell cultures and HRP with yeast cells and cell cultures (Para. 0031, 0032), to increase oxidative activity and generate excess ROS before the step of adding an antioxidant sample. This step in the process of the invention is not taught or suggested in any of the prior art references. Accordingly, it is respectfully submitted that the 103 rejection of Claims 1-3, 9-16 and 4-8 should be withdrawn.

With regard to the examiners rejection of Claim 17 under 35 U.S.C. 112, first paragraph, the examiner states that, "The specification does not enable one having ordinary skill in the art to make an in vitro model mimicking the gastrointestinal tract."

The specification recites that a biological sampling system using the OASP of the invention is broken down into five vessels representing five segments of the

gastrointestinal (GI) tract (Paragraph 0057), and that a sample is drawn from a select vessel representative of a specific step in the digestive process (Paragraph 0056). In addition, the specification recites that the first vessel simulates the acidic environment of the stomach (Paragraph 0058) and that solutions are added to the small intestine vessel to simulate the small intestine environment (Paragraph 0059).

The specification also recites that vessels contain ingredients that serve to mimic the environment in a segment of the GI tract (Paragraph 57), and additionally recites statements of mimicking the alkaline environment of the GI tract (Paragraph 58) and mimicking the environment and pH of the small intestine (Paragraph 0060).

Accordingly, as the terms mimicking, simulating, and representing are used interchangeably throughout the specification, it is clear that these terms are equivalent in the context of the teaching of the specification, and there is no intent to present an exact model of the gastrointestinal tract. As such, the process of the invention it not intended to enable one having ordinary skill in the art to make an exact model of the gastrointestinal tract, as stated by the examiner.

Regarding the examiners rejection of Claims 1-17 under 35 U.S.C. 112, second paragraph:

Claims 1 and 4 have been amended to delete the phrase, "assaying the free radical scavenging effectiveness of the nutritional formulation".

Claim 9 has been amended to clarify the difference between the first and second samples and how measurements relate to the free radical scavenging efficiency of the nutritional formulations, as recited in the specification.

Claims 10-16 have been amended to reflect the changes to Claim 9.

Claim 15 has been amended to delete the phrase, "proven to have antioxidant activity".

Claim 17 has been amended to particularly point out the method steps by which the solution is assayed, and reference to the terms, "mimic the environment" and "the gastrointestinal tract" has been deleted.

Claims 18-20 have been added to reflect the changes to Claim 17.